

## **Analysis of Anabolic Steroids**

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### **1. Background**

Steroids come in two forms which are anabolic and androgenic. Anabolic steroids promote muscle growth, enhance athletic or other physical performance and improve physical appearance. Androgenic steroids promote the development of sex characteristics and maintain traits such as body hair, deepening of the voice and baldness. Regardless of their specific physiological role, all steroids contain the same basic cyclopentanoperhydrophenanthrene ringed nucleus. The variation on the basic nucleus determines the specific steroid and its properties. Steroids are most commonly found as injectable oils, tablets and capsules. Other forms may include powders, creams, liquid drops and transdermal patches.

### **2. Objective**

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain an anabolic steroid.

### **3. Scope**

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

{ DATE \@ "M/d/yyyy" }

#### **4. Responsibility**

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

#### **1. Related Documents**

Cole, Michael, "The Analysis of Controlled Substances," London: John Wiley & Sons Ltd., 2003  
Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.  
Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3<sup>rd</sup> ed., 6 vols., New York: CRC Press, 2006.  
Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2<sup>nd</sup> ed., London: The Pharmaceutical Press, 1986.  
Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3<sup>rd</sup> ed., London: The Pharmaceutical Press, 2004.  
Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.  
Scientific Working Group for the Analysis of Seized Drug Recommendation, 6<sup>th</sup> ed., "Part III A & B, Methods of Analysis/Sampling of Seized Drug for Qualitative Analysis," July 2011

#### **6. Definitions**

GC: Gas Chromatography

GC/MS: Gas Chromatography/Mass Spectrometry

Gross Weight: The weight of both the substance and its container.

Net Weight: The weight of the substance only.

#### **7. Supplies, Equipment & Reagents**

##### **Supplies**

Culture tubes

Syringes with needle

Scissors

Spatula

Pasteur pipette

Porcelain spot plate

Pestle

Stirring rod

Various Class A glassware

Beakers  
Graduated cylinders  
Volumetric flask

Weighing dish  
Weighing paper  
GC vials with Teflon caps

**Equipment**

Analytical Balance  
GC with FID  
GC/MS

**Reagents**

Methanol  
Chloroform  
Acetone  
Deionized water  
Formaldehyde  
Sulfuric acid  
Cobalt thiocyanate  
Sodium molybdate  
Selenous acid  
Cobaltous acetate tetrahydrate  
Glacial acetic acid  
Isopropylamine  
Cocaine Hydrochloride  
Codeine Phosphate  
Anastrozole  
Androstenediol  
Boldenone Undecylenate  
Bolandirol  
1-Dehydrotestosterone  
Drostanolone  
Drostanolone Propionate  
Estrone  
17 $\alpha$ -Estradiol  
Mesterolone  
Methandrostenolone  
Methenolone  
Methyldihydrotestosterone  
17 $\alpha$ -Methyltestosterone  
Nandrolone  
Nandrolone Decanoate  
Nandrolone Phenpropionate  
Norgestrel  
Ondansetron Hydrochloride Dihydrate  
Oxandrolone  
Oxymethalone  
Stanozolol

Testosterone  
Testosterone Decanoate  
Testosterone Enanthate  
Testosterone Phenylpropionate  
Testosterone Propionate  
Trenbolone Acetate  
Trenbolone Enanthate

## 8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses. Lab coats are to be worn at all times in the unit, unless performing administrative duties.

## 9. Reagent Preparation

### Anastrozole Standard

Dissolve 2.0mg of anastrozole and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

### Androstenediol Standard

Dissolve 2.0mg of androstenediol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

### Boldenone Undecylenate Standard

Dissolve 4.0mg of boldenone undecylenate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

### Bolandioli Standard

Dissolve 0.2mg of bolandioli and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

### 1-Dehydrotestosterone Standard

Dissolve 2.0mg of 1-dehydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

### Drostanolone Standard

Dissolve 2.0mg of drostanolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

### Drostanolone Propionate Standard

Dissolve 2.0mg of drostanolone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

### Estrone Standard

Dissolve 2.0mg of estrone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17 $\alpha$ -Estradiol Standard

Dissolve 2.0mg of 17 $\alpha$ -estradiol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Mesterolone Standard

Dissolve 2.0mg of mesterolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methandrostenolone Standard

Dissolve 2.0mg of methandrostenolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methenolone Standard

Dissolve 0.2mg of methenolone and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

Methyldihydrotestosterone Standard

Dissolve 1.0mg of methyldihydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17 $\alpha$ -Methyltestosterone Standard

Dissolve 2.0mg of 17 $\alpha$ -methyltestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Standard

Dissolve 2.0mg of nandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Decanoate Standard

Dissolve 2.0mg of nandrolone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Phenpropionate Standard

Dissolve 2.0mg of nandrolone phenpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Norgestrel Standard

Dissolve 2.0mg of norgestrel and bring to volume with 1.8mL of acetone. Mix the solution until completely dissolved.

Ondansetron Hydrochloride Dihydrate Standard

Dissolve 2.0mg of ondansetron hydrochloride dihydrate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxandrolone Standard

Dissolve 2.0mg of oxandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxymethalone Standard

Dissolve 1.0mg of oxymetholone and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Stanozolol Standard

Dissolve 4.0mg of stanozolol and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Testosterone Standard

Dissolve 2.0mg of testosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Decanoate Standard

Dissolve 2.0mg of testosterone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Enanthate Standard

Dissolved 2.0mg of testosterone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Phenylpropionate Standard

Dissolve 2.0mg of testosterone phenylpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Propionate Standard

Dissolve 2.0mg of testosterone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Trenbolone Acetate Standard

Dissolve 2.0mg of trenbolone acetate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Trenbolone Enanthate Standard

Dissolve 2.0mg of trenbolone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Cobalt Thiocyanate Reagent

Dissolve 2.0g of cobalt thiocyanate in 100mL of deionized water. Mix the solution until completely dissolved.

Marquis Reagent

Dilute 10mL of 37% formaldehyde solution in 90mL of concentrated sulfuric acid. While stirring, slowly add the concentrated sulfuric acid to the formaldehyde solution. Allow the solution to cool completely.

Froedhde's Reagent

Dissolve 0.5g of sodium molybdate in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Mecke's Reagent

Dissolve 1.0g of selenous acid in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Dilly Koppanyi Reagent

Dilly

Dissolve 0.1g of cobaltous acetate tetrahydrate in 100mL of methanol. Then add 0.2mL of glacial acetic acid to the solution. Mix the solution until completely dissolved.

Isopropylamine

Dilute 5mL of isopropylamine in 100mL of methanol. Mix the solution completely.

2.8N Hydrochloric Acid Reagent

Dilute 92.6mL of 12.1N hydrochloric acid in 400mL of deionized water. Mix the solution completely.

20% Acetic Acid Reagent

Dilute 100mL of glacial acetic acid in 400mL of deionized water. Mix the solution completely.

Cocaine/Codeine Standard (QC Mix)

Dissolve 10.0 mg of cocaine hydrochloride and 10.0mg of codeine phosphate and bring to volume with 10mL of methanol. Mix the solution until completely dissolved.

## 10. Procedure

- A. Document observations on the Drug Analysis Form noting the number, type (e.g. liquid, gel/cream, powder, tablets, etc.), color, viscosity and marking of all items.
- B. Document if the sample has been tampered or intact.
- C. Androgenic steroids or human growth hormones should not be analyzed and will be documented as Not Tested.
- D. Obtain the weight of the sample.
  - i. For liquid, cream/gel and residual patches, only a gross weight should be documented.
  - ii. For powders and tablets, both a gross weight and net weight should be documented.
- E. Sampling Plan
  - i.
- F. Sample Preparation
  - i. For powders, no preparation is needed for color test. For GC or GC/MS analysis, place 1-2 mg of sample into a labeled test tube and dissolve with 1-2mL of methanol. Vortex and let stand for ½ hr.
  - ii. For tablets, use ¼ of the tablet and crush into a powdered form. For GC or GC/MS analysis, place 1-2mg of the sample into a labeled test tube and dissolve with 1-2mL of methanol. Vortex and let stand for ½ hr.
  - iii. For liquids, extract ½ mL of the liquid using a syringe and place into a labeled test tube. For GC or GC/MS analysis, place 2-4 drops of the sample into a labeled test tube and dilute with 1-2 mL of methanol. Vortex and let stand for ½ hr.

- iv. For gels or creams, remove a 3-4mg amount of sample and place into a labeled test tube. For GC or GC/MS analysis, place 1-2mg of the sample into a labeled test tube and dissolve with 1-2 mL of methanol. Vortex and let stand for ½ hr.
- v. For transdermal patches, cut ½ of the patch into smaller pieces and place into a labeled test tube. For GC or GC/MS analysis, place ½ of the cut pieces of the sample into a labeled test tube and dissolve with ½ - 1mL of methanol. Vortex and let stand for ½ hr.

G. Color Tests

- i. The color test consists of five reagents, which are cobalt thiocyanate, marquis, froehde's, mecke's and dilly koppanyi.
- ii. Place a couple of drops of cobalt thiocyanate, marquis, froehde's, mecke's reagents into individual wells on a porcelain spot plate.
- iii. Add a small amount of sample (1-2mg of powder or 1-2 drops of liquid) to each well.
- iv. For dilly koppanyi color test, add a small amount of sample (1-2mg of powder/gel or 1-2 drops of liquid) to a labeled test tube. Then add equal amounts (1ml) of dilly and isopropylamine reagents respectively to the test tube.
- v. Note any color change or reaction. The results will be recorded on the Drug Analysis Form by documenting the actual color/s observed. Negative observations will be recorded by stating no reaction present or no color change.

H. Gas Chromatography

- i. The methanolic extract from section (F) can be used for the GC analysis. Pipette the extract into a labeled GC vial and cap tightly.
- ii. Initiate auto sampler sequence using the appropriate method running a blank solvent between each unknown sample and reference standard/s.
- iii. Compare retention time of the each sample with the reference standard/s. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.
- iv. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

I. Gas Chromatography/Mass Spectrometry

- i. Confirmatory analysis can be performed using the labeled GC vial from the previous section (H).
- ii. Initiate auto sampler sequence using the SCREEN method running a blank solvent between each unknown sample.
- iii. Using the chromatogram and ion spectra, determine whether or not an illicit narcotic is present.
- iv. Document the results on the MS Tracking Sheet. For positive GC/MS, document which illicit narcotic is present and its concentration. For negative results, document observations by stating negative on the results section.
- v. If an illicit narcotic is present, then initiate auto sampler sequence using the appropriate method running a blank solvent between each unknown sample and the reference standard/s.
- vi. Compare retention time and ion spectra of the each sample with the reference standard/s.
- vii. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

## **11. Documentation**

- A. All results will be documented on the Drug Analysis Form.
- B. All raw data will be generated and filed according to the laboratory policy.
- C. A certificate of analysis will be generated for each lab number which will document the results.

## **12. Attachments**